Tumor necrosis factor α attenuates interferon α signaling in the liver: involvement of SOCS3 and SHP2 and implication in resistance to interferon therapy[1]

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SPECIFIC AIM

The present study demonstrates that tumor necrosis factor α (TNF-α) suppresses interferon α (IFN-α) signaling and induces expression of suppressor of cytokine signaling 3 (SOCS3) and SH2 containing protein-tyrosine phosphatase 2 (SHP2) in the liver, suggesting that TNF-α may be involved in resistance to IFN-α therapy and could be a potential therapeutic target for improving the efficacy of IFN-α therapy.

PRINCIPAL FINDINGS

1. TNF-α inhibits IFN-α-activated STAT1 in the liver *in vivo*

It has been reported that patients with high levels of TNF-α respond poorly to IFN-α therapy, suggesting that TNF-α may be involved in resistance to IFN-α therapy. To test this hypothesis, effects of TNF-α on IFN-α signaling in the liver in vivo were studied. As phosphorylation on STAT1 at Tyr (701) is essential for dimerization and DNA binding induced by IFN-α, phosphorylation at this site is an excellent marker for IFN-α signaling pathway activation. As shown in **Fig. 1*A***, administration of IFN-α stimulated both STAT1α (91 kDa) and STAT1β (84 kDa) tyrosine (Tyr (701) phosphorylation. Injection of TNF-α markedly suppressed IFN-α-activated STAT1 but increased STAT1 protein expression. Furthermore, we have demonstrated that injection of TNF-α alone markedly induced STAT1 protein expression (Fig. 1*B*) and various concentrations of TNF-α markedly inhibited IFN-α-activated STAT1 tyrosine phosphorylation and induced STAT1 protein expression in the liver (Fig. 1*C*). TNF-α inhibition of IFN-α signaling in the liver could be observed at very low doses such as 0.25 ng/g body weight. Taken together, these findings suggest that TNF-α induces STAT1 protein expression but inhibits IFN-α-activated STAT1 tyrosine phosphorylation in the liver *in vivo*.

2. Evidence for the involvement of suppressor of SOCS3 in TNF-α-mediated inhibition of IFN-α signaling in the liver

TNFα inhibition of IFN-α-activated STAT1 in the liver was only observed 2 h after injection as demonstrated in Fig. 1*A*, suggesting that this inhibition may require new protein synthesis. To further identify what kinds of inhibitory proteins were involved in TNF-α-mediated inhibition of IFN-α signaling in the liver, the expression of a suppressor of the cytokine signaling (SOCS) family of proteins and a protein inhibitor of the activated STAT (PIAS) family of proteins was examined. Normal mouse liver expresses very low levels of SOCS1, SOCS2, SOCS3, PIAS1, and PIAS3. Injection of TNF-α rapidly induced SOCS3 mRNA expression, with the peak effect occurring at 2 and 4 h, whereas SOCS1, SOCS2, PIAS1, and PIAS3 were not significantly induced. High levels of CIS mRNA were detected in normal liver and were unaffected after injection of TNF-α. These findings suggest that TNF-α specifically induces SOCS3 mRNA expression in the liver. Moreover, overexpression of SOCS3 markedly attenuated IFN-α-induced reporter gene expression. These findings suggest that induction of SOCS3 may be, at least in part, responsible for TNF-α-mediated inhibition of IFN-α signaling in the liver.

3. TNF-α stimulates expression of SHP2 in the liver

Protein tyrosine phosphatase (PTP) is another important pathway involved in suppression of IFN-α signaling. To test whether PTP is involved in TNF-α-mediated inhibition of IFN-α signaling in the liver, expression of 10 different PTPs (KAP, LAR, MPK2, PTP1B, SHP1, SHP2, RPTPα, RPTPβ, SIRPα1, VHR) were examined

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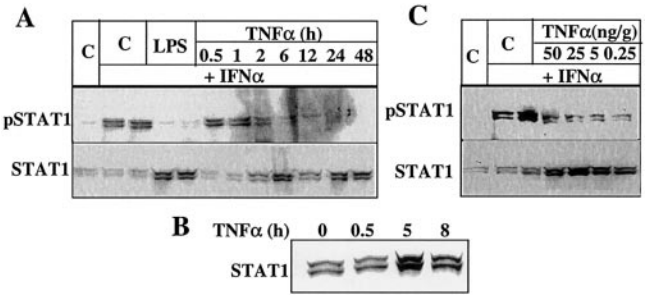

Figure 1. TNF-α inhibits IFN-α-activated STAT1 in the liver in vivo. *A)* Mice were intravenously (i.v.) injected with LPS (30 ng/g bwt) for 8 h or with TNF-α (50 ng/g bwt) for various periods as indicated, followed by injecting with IFN-α (40 ng/g bwt) for 30 min. *B)* Mice were injected with TNF-α (50 ng/g bwt) for various times as indicated. *C)* Mice were injected with various concentrations of TNF-α as indicated for 8 h, followed by injecting with IFN-α (40 ng/g bwt) for 30 min. Whole tissue extracts from the liver in panels *A–C* were subjected to Western blotting analysis by using anti-phospho-STAT1 (Tyr (701) or anti-STAT1 antibodies. Both STAT1α (91 kDa) and STAT1β (84 kDa) were detected.

in TNF-α-treated mouse liver. After injection of TNF-α, SHP2 was markedly induced in the liver, whereas other PTPs were not significantly induced. These findings suggest that TNF-α specifically induces SHP2 protein expression in the liver. Furthermore, coprecipitation experiments showed that both JAK1 and TYK2 coprecipitated with SHP2 protein from normal liver without TNF-α treatment, suggesting that SHP2 and JAKs can specifically associate and form complexes in vivo. TNF-α treatment for 1 h markedly enhanced association of SHP-2 with JAK1 or TYK2. This increase is probably due to elevation of SHP2 protein expression. Moreover, IFN-α-induced reporter gene expression in HepG2 cells was markedly inhibited by transfection of SHP2 expression vector and completely suppressed by cotransfection of SOCS3 and SHP2 expression vectors. Therefore, induction of SHP2 protein expression and an increase in the association of SHP2 with JAKs may contribute at least in part to TNF-α-mediated inhibition of IFN-α signaling in the liver *in vivo*.

4. IL-6 is responsible for TNF-α induction of STAT1 protein expression but not for induction of SOCS3 and SHP2 in the liver

The above data clearly indicate that TNF-α induction of SOCS3 is involved in TNF-α inhibition of IFN-α signaling in the liver *in vivo*; however, treatment of either primary hepatocyte or HepG2 cells *in vitro* with TNF-α did not induce SOCS3 expression or inhibit IFN-α signaling. This suggests that TNF-α-induction of SOCS3 and inhibition of IFN-α signaling in the liver *in vivo* may be mediated by other factors. It has been reported that TNF-α stimulates interleukin 6 (IL-6) gene expression in the liver and IL-6 markedly stimulates SOCS3 expression in primary rat hepatocytes. Thus, we hypothesized that IL-6 may be responsible for TNF-α-mediated induction of SOCS3 mRNA and STAT1 protein expression in the liver. To test this hypothesis, TNF-α was administered into IL-6 knockout (IL-6 −/−) mice and control (IL-6+/+) mice. Injection of TNF-α markedly induced SOCS3 mRNA, STAT1 protein, and SHP2 protein expression in the liver of IL-6 (+/+) mice. In IL-6 (−/−) mice, TNF-α induction of SHP2 remained unchanged, induction of SOCS3 mRNA was slightly but not significantly reduced, whereas induction of STAT1 protein was completely abolished. These findings suggest that IL-6 is responsible for TNF-α-induction of STAT1 protein expression, but not for induction of SOCS3 and SHP2 in the liver.

5. TNF-α is involved in acute liver injury- and inflammation-mediated inhibition of IFN-α signaling in the liver

Since liver injury and inflammation are known to induce TNF-α expression in the liver, we wondered whether liver injury and inflammation might inhibit IFN-α by a TNF-α-dependent mechanism. To test this hypothesis, a well-established liver injury and inflammation model induced by CCl4 was used. As shown in **Fig. 2*A, B***, injection of CCl4 markedly induced hepatic TNF-α mRNA expression and attenuated IFN-α-activated STAT1 in the liver with the strongest inhibition at doses of 0.01 mg/kg and 0.05 mg/kg of CCl4, despite increased STAT1 protein expression. It is well known

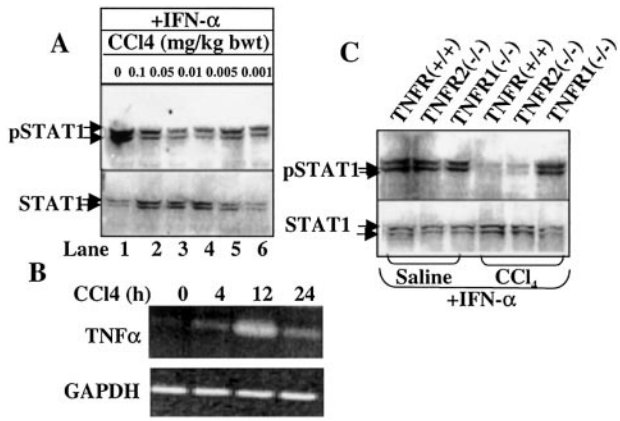

Figure 2. TNF-α is involved in acute liver injury- and inflammation-mediated inhibition of IFN-α-activated STAT1 in the liver. *A)* Mice were gavaged with various concentrations of CCl4 as indicated for 8 h, followed by injecting (i.v.) with IFN-α (40 ng/g bwt) for 30 min. Whole tissue extracts from the liver were subjected to Western blotting analysis by using anti-phospho-STAT1 (Tyr701) or anti-STAT1 antibodies. *B)* Mice were gavaged with CCl4 (10 ng/g) for 4, 12, or 24 h. Total RNA was isolated from the liver and subjected to RT-PCR by using TNF-α or GAPDH primers. *C)* Control mice [TNF receptor (TNFR) (+/+)], TNFR1 knockout mice (−/−), and TNFR2 knockout mice (−/−) were gavaged with saline or CCl4 (10 ng/g) for 8 h, followed by injecting with IFN-α (40 ng/g bwt) for 30 min. Whole tissue extracts from the liver were subjected to Western blotting analysis by using anti-phospho-STAT1 (Tyr701) or anti-STAT1 antibodies. Both STAT1α (91 kDa) and STAT1β (84 kDa) were detected.

that TNF-α is elevated with liver injury and inflammation. To determine whether TNF-α is involved in acute liver injury-mediated suppression of IFN-α signaling in the liver, TNF-α type 1 (TNFR1) and type 2 (TNFR2) receptor knockout mice were used. As shown in Fig. 2*C*, administration of CCl4 attenuated IFN-α-activated STAT1 in the livers of TNFR (+/+) mice and TNFR2 (−/−) mice, but not in the livers of TNFR1 (−/−) mice. These findings suggest that activation of TNFR1 by TNF-α is involved in liver injury-mediated suppression of IFN-α-activated STAT1 in the liver.

CONCLUSIONS AND SIGNIFICANCE

Here we demonstrate for the first time that TNF-α, which is greatly elevated in the serum of patients with viral hepatitis and other liver diseases, inhibits IFN-α signaling in the liver *in vivo*. It has been reported that patients with high expression of TNF-α in the liver or in the mononuclear cells respond poorly to IFN-α therapy. Taken together, both basic research and clinical data suggest that TNF-α is involved in resistance to IFN-α therapy. Furthermore, we demonstrate that SOCS3 and SHP2 are involved in TNF-α-mediated inhibition of IFN-α signaling in the liver *in vivo*. To best interpret these findings, we proposed a model shown in **Fig. 3** to explain resistance to IFN-α therapy caused by advanced liver injury. In this model, viral hepatitis → liver injury and advanced liver disease → high levels of TNF-α → high levels of SOCS3 and SHP2 expression in the liver → blocking IFN-α signaling → resistance to IFN-α therapy. The rationale for this model is presented in the discussion that follows. It is well known that levels of TNF-α are greatly elevated in the serum, the mononuclear cells, and the liver of viral hepatitis patients. In this paper, we clearly demonstrate that TNF-α inhibits IFN-α signaling in the liver *in vivo*. First, injection of synthetic TNF-α markedly inhibited IFN-α-induced STAT1 tyrosine phosphorylation in the liver (Fig. 1). Second, CCl4-mediated suppression of IFN-α signaling in the liver is abolished in TNFR1 (−/−) knockout mice (Fig. 2). Moreover, TNF-α has been implicated in resistance to IFN-α therapy in clinical viral hepatitis patients. These findings suggest that TNF-α may be involved in resistance to IFN-α therapy by inhibiting IFN-α signaling in the liver, and high levels of TNF-α in alcoholic and cirrhotic patients may contribute at least in part to the poor IFN response in these patients.

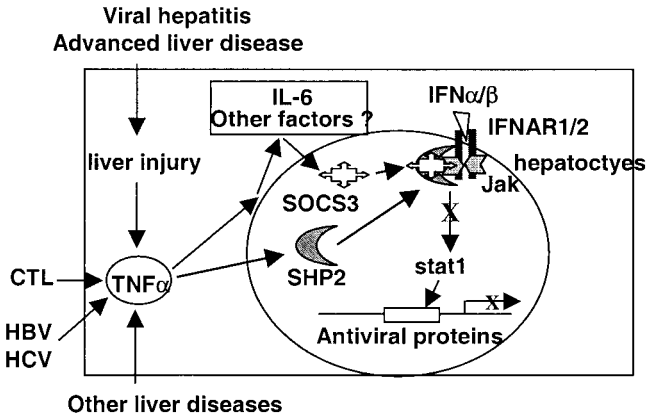

Figure 3. Schematic diagram. A model for TNF-α-mediated inhibition of IFN-α signaling in the liver *in vivo*. TNF-α is greatly elevated in patients with viral hepatitis and other liver diseases. TNF-α stimulates expression of SHP2 and SOCS3 proteins in the liver. SHP2 and SOCS3 bind to JAK-IFNAR and form IFNAR-JAK-SOCS3-SHP2 complexes, followed by inhibiting IFN-α-induced signaling and expression of antiviral proteins.

Three families of proteins have been implicated in down-regulating the JAK-STAT signaling pathway: *1)* SOCS; *2)* PIAS; *3)* PTPs. Our data showed that injection of TNF-α induced expression of SOCS3 and SHP2, but not other SOCSs and PTPs, in the liver. Overexpression of SOCS3 and SHP2 inhibited IFN-α signaling in HepG2 cells, HeLa cells, and MCF-7 cells, suggesting that induction of SOCS3 and SHP2 may be involved in TNF-α-mediated inhibition of IFN-α signaling in the liver *in vivo*. Finally, several clinical implications can be deduced from these studies: *1)* TNF-α inhibits IFN-α signaling in the liver *in vivo*, which may, at least in part, contribute to resistance to IFN-α therapy in the patients with high levels of TNF-α; *2)* TNF-α could be a potential therapeutic target for improving the efficacy of IFN-α therapy. For example, TNF-α antibody or antisense oligonucleotide could be used in nonresponder viral hepatitis patients with high levels of TNF-α to improve the efficacy of IFN-α therapy; and *3)* SOCS3 may be another potential therapeutic target for improving response to IFN-α in viral hepatitis patients. FJ